

## FINAL REPORT

**Test Facility Study No. 514035**

### **Determination of Physico-Chemical Properties of MLA-3202**

- **Hydrolysis as a function of pH**
- **Adsorption coefficient**

**SPONSOR:**  
Chemtura Corporation  
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USA

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**1. STATEMENT OF GLP COMPLIANCE**

Charles River Den Bosch, 's-Hertogenbosch, The Netherlands

All phases of this study performed by the test facility were conducted in compliance with the following GLP regulations:

- OECD Principles of Good Laboratory Practice, adopted on 26 November 1997, C(97)186/Final;
- EC Council Directive 2004, 2004/10/EC, February 11, 2004, Official Journal of February 20, 2004.

Except for the following:

- The characterization of the test item supplied by the sponsor was conducted in a GLP environment.

The data generated and reported are considered to be valid.

Charles River Den Bosch

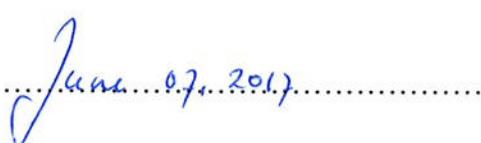


Signature: .....

Name: M.J.C. Brekelmans, MSc.

Title: Study Director

Date: .....



**2. TEST FACILITY QUALITY ASSURANCE STATEMENT**

Charles River Den Bosch, 's-Hertogenbosch, The Netherlands

Study title: Determination of physico-chemical properties of MLA-3202.

This report was inspected by the Charles River Den Bosch Quality Assurance Unit (QAU) according to the Standard Operating Procedure(s).

The reported method and procedures were found to describe those used and the report reflects the raw data.

During the on-site process inspections, procedures applicable to this type of study were inspected.

The dates of Quality Assurance inspections are given below:

**Project** 514035

Type of Inspections	Phase/Process	Start Inspection date	End Inspection date	Reporting date to TFM and SD*
<b>Study</b>	Study Plan	23-May-2016	23-May-2016	23-May-2016
	Study Plan Amendment 01	21-Oct-2016	21-Oct-2016	21-Oct-2016
	Study Plan Amendment 02	10-May-2017	10-May-2017	10-May-2017
	Report	29-May-2017	30-May-2017	30-May-2017
	Study Plan Amendment 03	07-June-2017	07-June-2017	07-June-2017
<b>Process</b>	<b>Analytical and physical chemistry</b>	05-Sep-2016	22-Sep-2016	30-Sep-2016
	Test Item Handling			
	Exposure			
	Observations/Measurements			
<b>Test Item Receipt</b>	Specimen Handling			
		21-Nov-2016	21-Nov-2016	21-Nov-2016
	Test Item Handling			
<b>Analytical and physical chemistry</b>	01-Dec-2016	28-Dec-2016	28-Dec-2016	
	Test Substance Handling			
	Exposure			
	Observations/Measurements			
<b>Test Substance Receipt</b>	Specimen Handling			
		06-Feb-2017	09-Feb-2017	13-Feb-2017
<b>Test Item Handling</b>				

<b>Analytical and physical chemistry</b>	06-Mar-2017	15-Mar-2017	23-Mar-2017
Test Item Handling			
Exposure			
Observations/Measurements			
Specimen Handling			
<b>Test Item Receipt</b>	08-May-2017	15-May-2017	16-May-2017
Test Item Handling			

\*TFM=Test Facility Management SD = Study Director

The facility inspection program is conducted in accordance with Standard Operating Procedure.

The review of the final report was completed on the date of signing this QA statement.

Charles River Den Bosch

Signature: .....

*A. Hout-Boudewijns*

Name: **Anita van Hout-Boudewijns, BSc**  
**Quality Assurance Auditor**

Date: .....

*07 June 2017*

**3. SUMMARY**

The results of the physico-chemical properties of the test item are given below.

Parameter	Guideline(s)	Result	Comment
Hydrolysis at pH 4	EC C.7 OECD 111 OPPTS 835.2120	Tests at 20, 50 and 60°C showed that the decrease in concentration observed is most probably due to adsorption or limited solubility in the buffer solution and not due to hydrolysis	
Hydrolysis at pH 7	EC C.7 OECD 111 OPPTS 835.2120	Tests at 20, 50 and 60°C showed that the decrease in concentration observed is most probably due to adsorption or limited solubility in the buffer solution and not due to hydrolysis	
Hydrolysis at pH 9	EC C.7 OECD 111 OPPTS 835.2120	Tests at 20, 50 and 60°C showed that the decrease in concentration observed is most probably due to adsorption or limited solubility in the buffer solution and not due to hydrolysis	
Adsorption coefficient	EC C.19 OECD 121	$\log K_{oc}$ : 5.4 (peak 1) $\log K_{oc}$ : 5.8 (peak 2) $\log K_{oc}$ : 6.1 (peak 3) $\log K_{oc}$ : 6.3 (peak 4) $\log K_{oc}$ : > 6.3 (peak 5) $\log K_{oc}$ : > 6.3 (peak 6)	HPLC method based on soil-adsorption-reference data; results for 6 major compounds

## 4. INTRODUCTION

### 4.1. Study schedule

Experimental starting date            06 September 2016  
Experimental completion date        19 May 2017

### 4.2. Purpose of the study

The purpose of the study was to determine the following physico-chemical properties for MLA-3202:

- Hydrolysis as a function of pH
- Adsorption coefficient

### 4.3. Retention of records and materials

Records and material pertaining to the study, which include study plan and amendments, raw data, specimens, except perishable specimens, and the final report will be retained in the archives of the test facility for a minimum of 5 years after the finalization of the report. After this period, the sponsor will be contacted to determine how the records and materials should be handled. The test facility will retain information concerning decisions made.

Perishable specimens (e.g. requiring refrigeration or freezing) will be discarded following evaluation in the study without further notice to the study sponsor.

A sample of the test item will be retained until expiry date or applicable retest date. After this period the sample(s) will be destroyed.

### 4.4. Responsible personnel

#### 4.4.1. Test facility

Study Director                          M.J.C. Brekelmans, MSc.

#### 4.4.2. Sponsor Representative

Study Monitor                          Audrey Batoon, Ph.D.

## 5. MATERIALS

### 5.1. Test item

#### 5.1.1. Test item information

Test item	207258/A
Identification	MLA-3202
Appearance	Clear amber-red liquid
Batch	RC-1045
Purity/Composition	UVCB
Test item storage	At room temperature
Stable under storage conditions until	17 February 2019 (expiry date)

#### 5.1.2. Study specific test item information

Purity/composition correction factor	No correction factor required
Test item handling	No specific handling conditions required
Chemical name (IUPAC), synonym or trade name	Amides, tallow, N,N-bis(2-hydroxypropyl)
CAS Number	1454803-04-3

### 5.2. Electronic systems for data acquisition

System control, data acquisition and data processing were performed using the following programs:

- Empower 3 database version 7.21 (Waters, Milford, MA, USA)
- MassLynx version 4.1 (Waters, Milford, MA, USA)

Temperature, relative humidity and/or atmospheric pressure during sample storage and/or performance of the studies was monitored continuously using the following programs:

- REES Centron Environmental Monitoring system version SQL 2.0 (REES Scientific, Trenton, NJ, USA)
- TestoComSoft version 3.4 (Testo, Lenzkirch, Germany)

### 5.3. List of deviations

#### 5.3.1. List of study plan deviations

1. During the hydrolysis main studies at 50 °C, the temperature was not controlled within  $\pm 0.5$  °C. Actual temperature was  $49.5 \pm 0.8$  °C.

Evaluation: The plots of the logarithm of the relative concentration as a function of time had correlation coefficients of at least 0.95. This shows that the slightly wider temperature range during the tests had no effect on the outcome of the study.

2. Recovery at start of the hydrolysis tests was not always within the criterion range of 70-110%. Evaluation: Because hydrolysis is calculated using the relative concentration, a slightly lower recovery at start of the test has no effect on the outcome of the study.

The study integrity was not adversely affected by the deviations.

#### 5.3.2. List of standard operating procedures deviations

Any deviations from standard operating procedures (SOPs) were evaluated and filed in the study file. There were no deviations from SOPs that affected the integrity of the study.

## 6. HYDROLYSIS AS A FUNCTION OF PH

### 6.1. Guidelines

- European Community (EC), EC no. 440/2008, Part C: Methods for the Determination of Ecotoxicity, Guideline C.7: "Degradation - Abiotic Degradation: Hydrolysis as a Function of pH", Official Journal of the European Union no. L142, May 31, 2008.
- Organization for Economic Co-operation and Development (OECD), OECD Guidelines for the Testing of Chemicals no. 111: "Hydrolysis as a Function of pH", April 13, 2004.
- United States Environmental Protection Agency (EPA), Fate, Transport and Transformation Test Guidelines no. OPPTS 835.2120: "Hydrolysis", October 2008.

### 6.2. Reagents

Water	Tap water purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA)
Methanol	Biosolve, Valkenswaard, The Netherlands
Ammonium acetate	Biosolve
Sodium acetate	Merck, Darmstadt, Germany
Acetic acid, 100%	Merck
Potassium <i>di</i> -hydrogenphosphate	Merck
Boric acid	Merck
Potassium chloride	Merck
Sodium azide	Merck
Sodium hydroxide, 1 N	Merck

All reagents were of analytical grade, unless specified otherwise.

### 6.3. Buffer solutions

Acetate buffer pH 4, 0.01 M	solution of 16.7% 0.01 M sodium acetate in water and 83.3% 0.01 M acetic acid in water. The buffer contains 0.0009% (w/v) sodium azide.
Phosphate buffer pH 7, 0.01 M	solution of 0.01 M potassium <i>di</i> -hydrogenphosphate in water adjusted to pH 7 using 1 N sodium hydroxide. The buffer contains 0.0009% (w/v) sodium azide.
Borate buffer pH 9, 0.01 M	solution of 0.01 M boric acid in water and 0.01 M potassium chloride in water adjusted to pH 9 using 1 N sodium hydroxide. The buffer contains 0.0009% (w/v) sodium azide.

### 6.4. Performance of the study

The rate of hydrolysis of the test item as a function of pH was determined at pH values normally found in the environment (pH 4-9).

#### 6.4.1. Preliminary test - Tier 1

The buffer solutions were filter-sterilised through a 0.2 µm FP 30/0.2 CA-S filter (Whatman, Dassel, Germany) and transferred into a sterile vessel. To exclude oxygen, nitrogen gas was purged through the solution for 5 minutes. The test item was spiked to the solutions at a target

concentration of 200 µg/L using a spiking solution in methanol. For each sampling time, duplicate sterile vessels under vacuum were filled with 6 mL test solution and placed in the dark in a temperature controlled environment at 49.6°C ± 0.2°C.

Note: the spiking volume was < 1% of the sample volume. Nominal concentrations were not corrected for the spiking volume.

The concentration of the test item in the test samples was determined immediately after preparation (t=0), after 2.4 hours and after 5 days. The samples taken at t=2.4 hours and at t=5 days were cooled to room temperature using running tap water. The samples were diluted in a 1:3 (v:v) ratio with methanol and analysed.

Blank buffer solutions containing a similar content of blank spiking solution were treated similarly as the test samples and analysed at t=0.

The pH of each of the test solutions (except for the blanks) was determined at each sampling time.

#### **6.4.2. Main study - Tier 2**

Test samples were prepared and treated similarly as during the preliminary test.

The concentrations of the test item were determined immediately after preparation (t=0) and at several sampling points after t=0.

Blank buffer solutions were treated similarly as the test samples and analysed at t=0.

The pH of each of the test solutions (except for the blanks) was determined at least at the beginning and at the end of the test.

The study was performed at the following temperatures:

pH code	Temperature I	Temperature II	Temperature III
pH 4	20.5°C ± 0.3°C	49.5°C ± 0.8°C	59.8°C ± 0.4°C
pH 7	20.5°C ± 0.3°C	49.5°C ± 0.8°C	60.0°C ± 0.4°C
pH 9	20.5°C ± 0.3°C	49.5°C ± 0.8°C	60.1°C ± 0.4°C

#### **6.4.3. Identification of hydrolysis products – Tier 3**

Tests at 20, 50 and 60°C showed that the decrease in concentration observed is most probably due to adsorption or limited solubility and not due to hydrolysis. Therefore Tier 3 testing was not applicable.

### **6.5. Analytical method**

#### **6.5.1. Analytical conditions**

Quantitative analysis was performed according to the analytical method as validated in project 511870.

Instrument	Acquity UPLC system (Waters, Milford, MA, USA)
Detector	Xevo TQ-S mass spectrometer (Waters)
Column	Acquity UPLC HSS Cyano, 100 mm × 2.1 mm i.d., dp = 1.8 µm (Waters)
Column temperature	40°C ± 1°C
Injection volume	5 µL

Mobile phase	10 mM Ammonium acetate in 70/30 (v/v) methanol/water
Flow	0.4 mL/min
MS detection	
Ionisation source	ESI <sup>+</sup>
Cone voltage	50 V
Acquisition	$m/z$ 398.2 → $m/z$ 134 (Collision energy 18 eV) $m/z$ 372.2 → $m/z$ 134 (Collision energy 16 eV) $m/z$ 396.2 → $m/z$ 134 (Collision energy 16 eV) $m/z$ 400.3 → $m/z$ 134 (Collision energy 18 eV)
Quantitation	$m/z$ 398.2 → $m/z$ 134

### 6.5.2. Preparation of the calibration solutions

#### Stock solutions

Stock solutions of the test item were prepared in methanol at concentrations of 2000 mg/L.

#### Calibration solutions

Five solutions with the test item in the concentration range of 200 - 30000 µg/L were prepared in methanol from two stock solutions. The solutions were diluted by a factor of 100 with 75/25 (v/v) methanol/buffer to obtain calibration solutions in the concentration range of 2 - 300 µg/L.

### 6.5.3. Sample injections

Calibration solutions were injected in duplicate. Test samples were analysed by single injection.

### 6.5.4. Calibration curves

Calibration curves were constructed using five concentrations. For each concentration, two responses were used. If necessary, two responses were excluded from the curve since the back calculated accuracy was > 15% from the nominal concentration. Linear regression analysis was performed using the least squares method with a 1/concentration<sup>2</sup> weighting factor. The coefficient of correlation (r) was > 0.99 for each curve.

## 6.6. Formulas

Response (R)	Peak area of the test item for the $m/z$ 398.2 → $m/z$ 134 transition [units]
Calibration curve	$R = a C_N + b$ where: $C_N$ = nominal concentration [µg/L] $a$ = slope [units × L/µg] $b$ = intercept [units]

Analysed concentration ( $C_A$ )

$$C_A = \frac{(R - b)}{a} \times d \text{ } [\mu\text{g/L}]$$

where:

 $d$  = dilution factor

Recovery

$$\frac{C_A}{C_N} \times 100\%$$

Degree of hydrolysis

$$\frac{\text{mean } C_0 - C_t}{\text{mean } C_0} \times 100\%$$

where:

 $C_0$  = concentration at  $t=0$  $C_t$  = concentration at  $t=5$  daysRelative concentration ( $C_r$ )

$$C_r = \frac{C_t}{\text{mean } C_0} \times 100\%$$

where:

 $C_0$  = concentration at  $t=0$  $C_t$  = concentration at  $t=x$  hours

Pseudo-first order curve

$$10 \log C_r = -a \times t + b$$

where:

 $t$  = time [hours] $a$  = slope [hours $^{-1}$ ] $b$  = interceptRate constant ( $k_{\text{obs}}$ )

$$k_{\text{obs}} = -a \times 2.303 \text{ [hours}^{-1}\text{]}$$

where:

2.303 is the conversion factor between natural and base 10 logarithms

Arrhenius equation

$$\ln k_{\text{obs}} = \frac{-E}{R \times T} + \ln A$$

where:

A plot of  $\ln k_{\text{obs}}$  versus  $1/T$  gives a linear relationship with a slope of  $-E/R$  and intercept  $\ln A$ Half-life time ( $t_{1/2}$ )

$$t_{1/2} = \frac{\ln 2}{k_{\text{obs}}} \text{ [hours]}$$

## 6.7. Results

### 6.7.1. Preliminary test - Tier 1

The analytical results of the preliminary test are given in [Table 1](#) and [Table 2](#).

A decrease in concentration of  $\geq 10\%$  was observed at pH 4, pH 7 and pH 9 after 5 days. According to the guideline, the higher Tier test was required if a reduction of  $>10\%$  is observed to determine the half-life time of the test item.

A small peak at the retention time of the test item was detected in the chromatograms of the blank buffer solutions. Maximum contribution to the test solutions at start of the test was  $<1\%$  based on peak area and therefore considered negligible.

The mean recovery of the test item containing buffer solutions at pH 4 at t=0 fell outside the criterion range of 70-110%. Because hydrolysis is calculated using the relative concentration, the slightly low recovery at pH 4 has no effect on the outcome of the study. The mean recoveries of the test item containing buffer solutions at pH 7 and pH 9 at t=0 fell within the criterion range of 70-110%.

**Table 1**  
**Preliminary test – hydrolysis of the test item at pH 4, pH 7 and pH 9**

pH code	Sampling time	Analysed concentration <sup>1</sup> [ $\mu\text{g/L}$ ]	Degree of hydrolysis [%]		Actual pH
			Individual	Mean	
pH 4	0 hours	119 131			4.1 4.1
	2.4 hours	66.2 79.1	47 37	42	4.1 4.1
	5 days	33.9 34.2	73 73	73	4.2 4.2
	0 hours	170 166			7.0 7.0
	2.4 hours	88.1 86.4	48 49	48	7.0 7.0
	5 days	41.7 38.7	75 77	76	7.0 7.0
pH 9	0 hours	170 167			9.2 9.2
	2.4 hours	116 105	31 38	34	9.2 9.2
	5 days	56.9 43.6	66 74	70	9.2 9.2

<sup>1</sup> Based on the response of the peak recorded at the transition m/z 398.2 → m/z 134

**Table 2**  
**Recoveries**

pH code	Nominal concentration <sup>1</sup> [µg/L]	Analysed concentration [µg/L]	Recovery [%]	Mean recovery [%]
pH 4	200	119	60	63
	200	131	66	
pH 7	200	170	85	84
	200	166	83	
pH 9	200	170	85	84
	200	167	84	

<sup>1</sup> Based on the response of the peak recorded at the transition m/z 398.2 → m/z 134

**6.7.2. Main study - Tier 2****pH 4**

The analytical results of the main study are given in [Table 3](#) to [Table 6](#). No test item was detected in the blank buffer solutions. The responses observed in some blanks were smaller than the responses in analytical blanks analysed in the same series.

The mean recovery of the of the test item containing buffer solutions at t=0 fell outside the criterion range of 70-110%. Because hydrolysis is calculated using the relative concentration, the low recovery has no effect on the outcome of the study.

**Table 3**  
**Main test – hydrolysis of the test item at pH 4 and 20°C**

Sampling time [hours]	Analysed concentration <sup>1</sup> [µg/L]	Relative concentration [%]	Logarithm relative concentration	Actual pH
0.00	121	101	2.00	4.1
0.00	119	99	2.00	4.1
21.65	56.1	47	1.67	4.1
21.65	52.2	43	1.64	4.1
46.12	61.8	51	1.71	4.1
46.12	71.4	59	1.77	4.1
48.17	75.7	63	1.80	4.1
48.17	92.4	77	1.89	4.0
50.20	60.5	50	1.70	4.0
50.20	81.0	67	1.83	4.1
143.80	77.3	64	1.81	4.1
143.80	82.6	69	1.84	4.1
213.55	59.7	50	1.70	4.1
213.55	58.9	49	1.69	4.1
287.75	73.1	61	1.78	4.1
287.75	73.0	61	1.78	4.1
455.78	66.6	55	1.74	4.1
455.78	63.9	53	1.73	4.1
551.93	63.1	53	1.72	4.1
551.93	61.9	52	1.71	4.1
626.42	70.3	58	1.77	4.1
626.42	62.5	52	1.72	4.1
721.97	67.3	56	1.75	4.1
721.97	82.2	68	1.84	4.1

<sup>1</sup> Based on the response of the peak recorded at the transition m/z 398.2 → m/z 134

**Table 4**  
**Main test – hydrolysis of the test item at pH 4 and 50°C**

Sampling time [hours]	Analysed concentration <sup>1</sup> [µg/L]	Relative concentration [%]	Logarithm relative concentration	Actual pH
0	109	102	2.01	4.1
0	104	98	1.99	4.0
2.03	55.0	52	1.71	4.1
2.03	71.9	68	1.83	4.1
3.12	61.4	58	1.76	4.1
3.12	80.2	75	1.88	4.1
22.67	56.3	53	1.72	4.1
22.67	46.3	44	1.64	4.1
27.67	45.6	43	1.63	4.1
27.67	47.8	45	1.65	4.1
48.05	42.4	40	1.60	4.1
48.05	43.7	41	1.61	4.1
72.28	38.0	36	1.55	4.1
72.28	37.9	36	1.55	4.2
98.67	33.7	32	1.50	4.2
98.67	35.6	33	1.52	4.2
169.20 <sup>2</sup>	28.6	27	1.43	4.1
169.20 <sup>2</sup>	31.0	29	1.46	4.1
215.85	26.2	25	1.39	4.1
215.85	28.1	26	1.42	4.1
261.82	24.4	23	1.36	4.1
261.82	26.8	25	1.40	4.1
335.80	23.9	22	1.35	4.1
335.80	24.3	23	1.36	4.1
406.03	21.1	20	1.30	4.1
406.03	17.4	16	1.21	4.1
503.78	15.2	14	1.16	4.1
503.78	19.4	18	1.26	4.1
720.95	15.3	14	1.16	4.1
720.95	15.0	14	1.15	4.1

<sup>1</sup> Based on the response of the peak recorded at the transition m/z 398.2 → m/z 134

<sup>2</sup> Due to a technical problem with the UPLC, samples taken and pretreated on 27 Feb 2017 had to be stored overnight at room temperature in the autosampler until analysis on 28 Feb 2017.

**Table 5**  
**Main test – hydrolysis of the test item at pH 4 and 60°C**

Sampling time [hours]	Analysed concentration <sup>1</sup> [µg/L]	Relative concentration [%]	Logarithm relative concentration	Actual pH
0.00	131	100	2.00	4.1
0.00	131	100	2.00	4.1
24.08	96.5	74	1.87	4.1
24.08	98.5	75	1.88	4.1
45.75	69.9	53	1.73	4.1
45.75	82.6	63	1.80	4.1
120.15	73.0	56	1.75	4.1
120.15	72.3	55	1.74	4.1
288.00	52.7	40	1.60	4.1
288.00	57.7	44	1.64	4.0
384.18	43.4	33	1.52	4.1
384.18	44.7	34	1.53	4.0
458.70	41.1	31	1.50	4.0
458.70	39.7	30	1.48	4.1
626.95	36.0	28	1.44	4.1
626.95	36.1	28	1.44	4.1
722.62	31.2	24	1.38	4.1
722.62	38.3	29	1.47	4.1

<sup>1</sup> Based on the response of the peak recorded at the transition m/z 398.2 → m/z 134

**Table 6**  
**Recoveries at pH 4**

Temperature (°C)	Nominal concentration <sup>1</sup> [µg/L]	Analysed concentration [µg/L]	Recovery [%]	Mean recovery [%]
20	200	121	60	60
	200	119	60	
50	200	109	54	53
	200	104	52	
60	200	131	65	65
	200	131	66	

<sup>1</sup> Based on the response of the peak recorded at the transition m/z 398.2 → m/z 134

For testing of pseudo-first order kinetics the mean logarithms of the relative concentrations between 10% and 90% were plotted against time. At all temperatures linear relationships were obtained though the correlation coefficient for the test at 20°C was poor.

The half-life times of the test item were determined according to the model for *pseudo-first* order reactions. Logarithms of the relative concentrations were correlated with time using linear regression analysis. [Figure 1](#) illustrates the regression curves and [Table 7](#) shows the statistical parameters.

**Table 7**  
**Statistical parameters of the regression curves at pH 4**

Temperature (°C)	Slope [hours <sup>-1</sup> ]	Intercept	Coefficient of correlation
20	$-1.36 \times 10^{-6}$	1.75	0.0055
50	$-6.31 \times 10^{-4}$	1.56	0.95
60	$-7.10 \times 10^{-4}$	1.86	0.93

The rate constant ( $k_{obs}$ ) and half-life time of the test item at each temperature was obtained and the Arrhenius equation was used to determine the rate constant and half-life time at 25°C (see [Table 8](#)).

**Table 8**  
**Rate constants ( $k_{obs}$ ) and half-life time ( $t_{1/2}$ ) at pH 4**

Temperature [°C]	$k_{obs}$ [hours <sup>-1</sup> ]	$t_{1/2}$ [days]
20	$3.12 \times 10^{-6}$	9249
50	$1.45 \times 10^{-3}$	20
60	$1.64 \times 10^{-3}$	18

These results however are not as expected for hydrolysis. For hydrolysis, rate constants and slopes increase by a factor 2 to 3 with a 10°C increase in temperature. This was not observed.

Based on the relatively low recovery at start of the tests and the steep decrease in concentration during the first hours of the tests at 50 and 60°C, it is expected that the decrease in concentration observed in the various tests at pH 4 is most probably due to adsorption and/or limited solubility and not due to hydrolysis. The amide bond in the various compounds present in MLA-3202 is expected to be stable at pH 4.

pH 7

The analytical results of the main study are given in [Table 9](#) to [Table 12](#). No test item was detected in the blank buffer solutions. The responses observed in some blanks were smaller than the responses in analytical blanks analysed in the same series.

In the test at 20°C, the mean recovery of the test item containing buffer solutions at t=0 fell outside the criterion range of 70-110%. Because hydrolysis is calculated using the relative concentration, low recovery has no effect on the outcome of the study. In the tests at 50 and 60°C, the mean recoveries of the test item containing buffer solutions at t=0 fell within the criterion range of 70-110%.

**Table 9**  
**Main test – hydrolysis of the test item at pH 7 and 20°C**

Sampling time [hours]	Analysed concentration <sup>1</sup> [µg/L]	Relative concentration [%]	Logarithm relative concentration	Actual pH
0.00	131	101	2.01	7.1
0.00	128	99	1.99	7.1
21.37	68.0	53	1.72	7.1
21.37	64.9	50	1.70	7.1
45.83	72.9	56	1.75	7.1
45.83	78.6	61	1.78	7.1
47.88	75.8	59	1.77	7.1
47.88	76.2	59	1.77	7.1
49.92	77.8	60	1.78	7.0
49.92	69.7	54	1.73	7.1
143.52	70.2	54	1.73	7.1
143.52	74.3	57	1.76	7.1
213.27	43.4	34	1.53	7.1
213.27	42.6	33	1.52	7.1
287.47	33.0	25	1.41	7.1
287.47	40.7	31	1.50	7.1
455.50	29.5	23	1.36	7.1
455.50	36.4	28	1.45	7.1
551.65	49.2	38	1.58	7.1
551.65	44.9	35	1.54	7.1
626.13	39.8	31	1.49	7.1
626.13	36.8	28	1.45	7.1
721.68	39.7	31	1.49	7.1
721.68	39.0	30	1.48	7.1

<sup>1</sup> Based on the response of the peak recorded at the transition m/z 398.2 → m/z 134

**Table 10**  
**Main test – hydrolysis of the test item at pH 7 and 50°C**

Sampling time [hours]	Analysed concentration <sup>1</sup> [µg/L]	Relative concentration [%]	Logarithm relative concentration	Actual pH
0	143	100	2.00	7.0
0	143	100	2.00	7.0
1.63	85.8	60	1.78	7.0
1.63	89.7	63	1.80	7.0
2.72	90.7	63	1.80	7.0
2.72	89.0	62	1.79	7.0
22.27	67.0	47	1.67	7.0
22.27	62.5	44	1.64	7.0
27.27	59.2	41	1.62	7.0
27.27	53.3	37	1.57	7.0
47.65	48.8	34	1.53	7.0
47.65	49.1	34	1.54	7.0
71.88	42.3	30	1.47	7.1
71.88	52.6	37	1.57	7.1
98.27	46.6	33	1.51	7.1
98.27	45.2	32	1.50	7.1
168.80 <sup>2</sup>	33.9	24	1.37	7.0
168.80 <sup>2</sup>	37.0	26	1.41	7.0
215.45	31.8	22	1.35	7.1
215.45	31.3	22	1.34	7.1
261.42	29.4	21	1.31	7.1
261.42	37.4	26	1.42	7.1
335.40	29.2	20	1.31	7.1
335.40	30.2	21	1.33	7.0
405.63	25.5	18	1.25	7.0
405.63	23.9	17	1.22	7.1
503.37	19.6	14	1.14	7.0
503.37	23.0	16	1.21	7.0
720.55	17.1	12	1.08	7.1
720.55	16.7	12	1.07	7.0

<sup>1</sup> Based on the response of the peak recorded at the transition m/z 398.2 → m/z 134

<sup>2</sup> Due to a technical problem with the UPLC, samples taken and pretreated on 27 Feb 2017 had to be stored overnight at room temperature in the autosampler until analysis on 28 Feb 2017

**Table 11**  
**Main test – hydrolysis of the test item at pH 7 and 60°C**

Sampling time [hours]	Analysed concentration <sup>1</sup> [µg/L]	Relative concentration [%]	Logarithm relative concentration	Actual pH
0.00	142	100	2.00	7.0
0.00	142	100	2.00	7.1
23.38	82.6	58	1.77	7.1
23.38	84.7	60	1.78	7.1
45.05	75.9	54	1.73	7.1
45.05	65.1	46	1.66	7.1
119.45	82.9	58	1.77	7.1
119.45	87.7	62	1.79	7.1
287.30	74.5	53	1.72	7.1
287.30	55.6	39	1.59	7.1
383.48	80.3	57	1.75	7.1
383.48	82.3	58	1.76	7.1
458.00	57.7	41	1.61	7.1
458.00	54.2	38	1.58	7.1
626.25	12.6	8.9	0.95 <sup>1</sup>	7.1
626.25	53.6	38	1.58	7.1
721.92	70.2	50	1.69	7.1
721.92	63.7	45	1.65	7.1

<sup>1</sup> Based on the response of the peak recorded at the transition m/z 398.2 → m/z 134

<sup>2</sup> Outlier; not used for further calculations.

**Table 12**  
**Recoveries at pH 7**

Temperature (°C)	Nominal concentration <sup>1</sup> [µg/L]	Analysed concentration [µg/L]	Recovery [%]	Mean recovery [%]
20	200	131	66	65
	200	128	64	
50	200	143	72	72
	200	143	71	
60	200	142	71	71
	200	142	71	

<sup>1</sup> Based on the response of the peak recorded at the transition m/z 398.2 → m/z 134

For testing of pseudo-first order kinetics the mean logarithms of the relative concentrations between 10% and 90% were plotted against time. At all temperatures linear relationships were obtained though the correlation coefficient for the tests at 20 and 60°C was poor.

The half-life times of the test item were determined according to the model for *pseudo-first* order reactions. Logarithms of the relative concentrations were correlated with time using linear regression analysis. [Figure 2](#) illustrates the regression curves and [Table 13](#) shows the statistical parameters.

**Table 13**  
**Statistical parameters of the regression curves at pH 7**

Temperature (°C)	Slope [hours <sup>-1</sup> ]	Intercept	Coefficient of correlation
20	$7.43 \times 10^{-6}$	1.48	0.023
50	$-7.05 \times 10^{-4}$	1.55	0.96
60	$-3.78 \times 10^{-4}$	1.77	0.43

The rate constant ( $k_{obs}$ ) and half-life time of the test item at each temperature was obtained and the Arrhenius equation was used to determine the rate constant and half-life time at 25°C (see [Table 14](#)).

**Table 14**  
**Rate constants ( $k_{obs}$ ) and half-life time ( $t_{1/2}$ ) at pH 7**

Temperature [°C]	$k_{obs}$ [hours <sup>-1</sup> ]	$t_{1/2}$ [days]
20	$-1.71 \times 10^{-5}$	-1687
50	$1.62 \times 10^{-3}$	18
60	$8.71 \times 10^{-4}$	33

These results however are not as expected for hydrolysis. For hydrolysis, rate constants and slopes increase by a factor 2 to 3 with a 10°C increase in temperature. This was not observed. Even a negative half-life time was obtained at 20°C due to a slight increase of concentration with time which is not possible in case of hydrolysis.

Based on the relatively low recovery at start of the tests and the steep decrease in concentration during the first hours of the tests at 50 and 60°C, it is expected that the decrease in concentration observed in the various tests at pH 7 is most probably due to adsorption and/or limited solubility and not due to hydrolysis. The amide bond in the various compounds present in MLA-3202 is expected to be stable at pH 7.

pH 9

The analytical results of the main study are given in [Table 15](#) to [Table 18](#). No test item was detected in the blank buffer solutions. The responses observed in some blanks were smaller than the responses in analytical blanks analysed in the same series.

In the test at 20°C, the mean recovery of the test item containing buffer solutions at t=0 fell outside the criterion range of 70-110%. Because hydrolysis is calculated using the relative concentration, low recovery has no effect on the outcome of the study. In the tests at 50 and 60°C, the mean recoveries of the test item containing buffer solutions at t=0 fell within the criterion range of 70-110%.

**Table 15**  
**Main test – hydrolysis of the test item at pH 9 and 20°C**

Sampling time [hours]	Analysed concentration <sup>1</sup> [µg/L]	Relative concentration [%]	Logarithm relative concentration	Actual pH
0.00	138	100	2.00	9.0
0.00	140	100	2.00	9.0
19.80	78.6	57	1.75	8.9
19.80	77.8	56	1.75	8.9
44.27	82.1	59	1.77	8.9
44.27	92.7	67	1.82	8.9
46.32	93.3	67	1.83	8.9
46.32	91.3	66	1.82	8.9
48.35	87.4	63	1.80	8.9
48.35	89.2	64	1.81	8.9
141.95	96.9	70	1.84	9.0
141.95	98.2	71	1.85	9.0
211.70	27.2	20	1.29	9.0
211.70	19.9	14	1.16	9.0
285.90	28.0	20	1.30	8.9
285.90	25.7	19	1.27	9.0
453.93	28.8	21	1.32	9.0
453.93	24.4	18	1.25	9.0
550.08	32.4	23	1.37	9.0
550.08	34.3	25	1.39	9.0
624.57	30.9	22	1.35	9.0
624.57	25.2	18	1.26	9.0
720.12	32.6	23	1.37	9.0
720.12	35.6	26	1.41	9.0

<sup>1</sup> Based on the response of the peak recorded at the transition m/z 398.2 → m/z 134

**Table 16**  
**Main test – hydrolysis of the test item at pH 9 and 50°C**

Sampling time [hours]	Analysed concentration <sup>1</sup> [µg/L]	Relative concentration [%]	Logarithm relative concentration	Actual pH
0	154	99	1.99	8.9
0	157	101	2.01	9.0
1.10	95.4	61	1.79	9.0
1.10	84.7	55	1.74	9.0
2.18	93.1	60	1.78	9.0
2.18	81.8	53	1.72	9.0
21.73	67.1	43	1.64	8.9
21.73	65.5	42	1.62	9.0
26.73	62.6	40	1.60	9.0
26.73	68.7	44	1.65	9.0
47.12	56.2	36	1.56	9.0
47.12	54.7	35	1.55	9.0
71.35	52.7	34	1.53	9.0
71.35	51.3	33	1.52	9.0
97.73	50.1	32	1.51	9.0
97.73	53.0	34	1.53	9.0
168.27 <sup>2</sup>	42.9	28	1.44	8.9
168.27 <sup>2</sup>	36.5	23	1.37	9.0
214.92	39.5	25	1.40	9.0
214.92	34.2	22	1.34	9.0
260.88	28.9	19	1.27	9.0
260.88	34.1	22	1.34	9.0
334.87	33.4	22	1.33	9.0
334.87	36.8	24	1.37	9.0
405.10	30.2	19	1.29	9.0
405.10	27.5	18	1.25	9.0
502.85	26.6	17	1.23	9.0
502.85	23.1	15	1.17	9.0
720.02	19.4	12	1.10	9.0
720.02	18.4	12	1.07	9.0

<sup>1</sup> Based on the response of the peak recorded at the transition m/z 398.2 → m/z 134

<sup>2</sup> Due to a technical problem with the UPLC, samples taken and pretreated on 27 Feb 2017 had to be stored overnight at room temperature in the autosampler until analysis on 28 Feb 2017.

**Table 17**  
**Main test – hydrolysis of the test item at pH 9 and 60°C**

Sampling time [hours]	Analysed concentration <sup>1</sup> [µg/L]	Relative concentration [%]	Logarithm relative concentration	Actual pH
0.00	163	101	2.00	8.9
0.00	160	99	2.00	8.9
21.63	77.4	48	1.68	8.9
21.63	96.1	59	1.77	8.9
43.30	75.7	47	1.67	9.0
43.30	75.2	47	1.67	9.0
117.70	75.8	47	1.67	9.0
117.70	75.1	46	1.67	9.0
285.55	82.2	51	1.71	9.0
285.55	78.3	48	1.69	9.0
381.73	62.3	39	1.59	9.0
381.73	58.8	36	1.56	9.0
456.25	68.7	43	1.63	9.0
456.25	57.2	35	1.55	9.0
624.50	50.6	31	1.50	9.0
624.50	49.4	31	1.49	9.0
720.17	53.5	33	1.52	9.0
720.17	61.1	38	1.58	9.0

<sup>1</sup> Based on the response of the peak recorded at the transition m/z 398.2 → m/z 134

**Table 18**  
**Recoveries at pH 9**

Temperature (°C)	Nominal concentration <sup>1</sup> [µg/L]	Analysed concentration [µg/L]	Recovery [%]	Mean recovery [%]
20	200	138	69	69
	200	140	70	
50	200	154	77	78
	200	157	79	
60	200	163	82	81
	200	160	80	

<sup>1</sup> Based on the response of the peak recorded at the transition m/z 398.2 → m/z 134

For testing of pseudo-first order kinetics the mean logarithms of the relative concentrations between 10% and 90% were plotted against time. At all temperatures linear relationships were obtained though the correlation coefficient for the tests at 20 and 60°C was poor.

The half-life times of the test item were determined according to the model for *pseudo-first* order reactions. Logarithms of the relative concentrations were correlated with time using linear regression analysis. [Figure 3](#) illustrates the regression curves and [Table 19](#) shows the statistical parameters.

**Table 19**  
**Statistical parameters of the regression curves at pH 9**

Temperature (°C)	Slope [hours <sup>-1</sup> ]	Intercept	Coefficient of correlation
20	$2.65 \times 10^{-4}$	1.18	0.69
50	$-6.86 \times 10^{-4}$	1.55	0.96
60	$-2.77 \times 10^{-4}$	1.71	0.84

The rate constant ( $k_{obs}$ ) and half-life time of the test item at each temperature was obtained and the Arrhenius equation was used to determine the rate constant and half-life time at 25°C (see [Table 20](#)).

**Table 20**  
**Rate constants ( $k_{obs}$ ) and half-life time ( $t_{1/2}$ ) at pH 9**

Temperature [°C]	$k_{obs}$ [hours <sup>-1</sup> ]	$t_{1/2}$ [days]
20	$-6.11 \times 10^{-4}$	-47
50	$1.58 \times 10^{-3}$	18
60	$6.37 \times 10^{-4}$	45

These results however are not as expected for hydrolysis. For hydrolysis, rate constants and slopes increase by a factor 2 to 3 with a 10°C increase in temperature. This was not observed. Even a negative half-life time was obtained at 20°C due to a slight increase of concentration with time which is not possible in case of hydrolysis.

Based on the relatively low recovery at start of the tests and the steep decrease in concentration during the first hours of the tests at 50 and 60°C, it is expected that the decrease in concentration observed in the various tests at pH 9 is most probably due to adsorption and/or limited solubility and not due to hydrolysis. The amide bond in the various compounds present in MLA-3202 is expected to be stable at pH 9.

## 6.8. Conclusion

The preliminary test (Tier 1) and main study (Tier 2) were performed for the determination of the rate of hydrolysis of MLA-3202 at pH values normally found in the environment (pH 4-pH 9).

The half-life times of the test item were:

pH 4		pH 7		pH 9	
Temperature [°C]	t <sub>1/2</sub> [days]	Temperature [°C]	t <sub>1/2</sub> [days]	Temperature [°C]	t <sub>1/2</sub> [days]
20	9249	20	-1687	20	-47
50	20	50	18	50	18
60	18	60	33	60	45

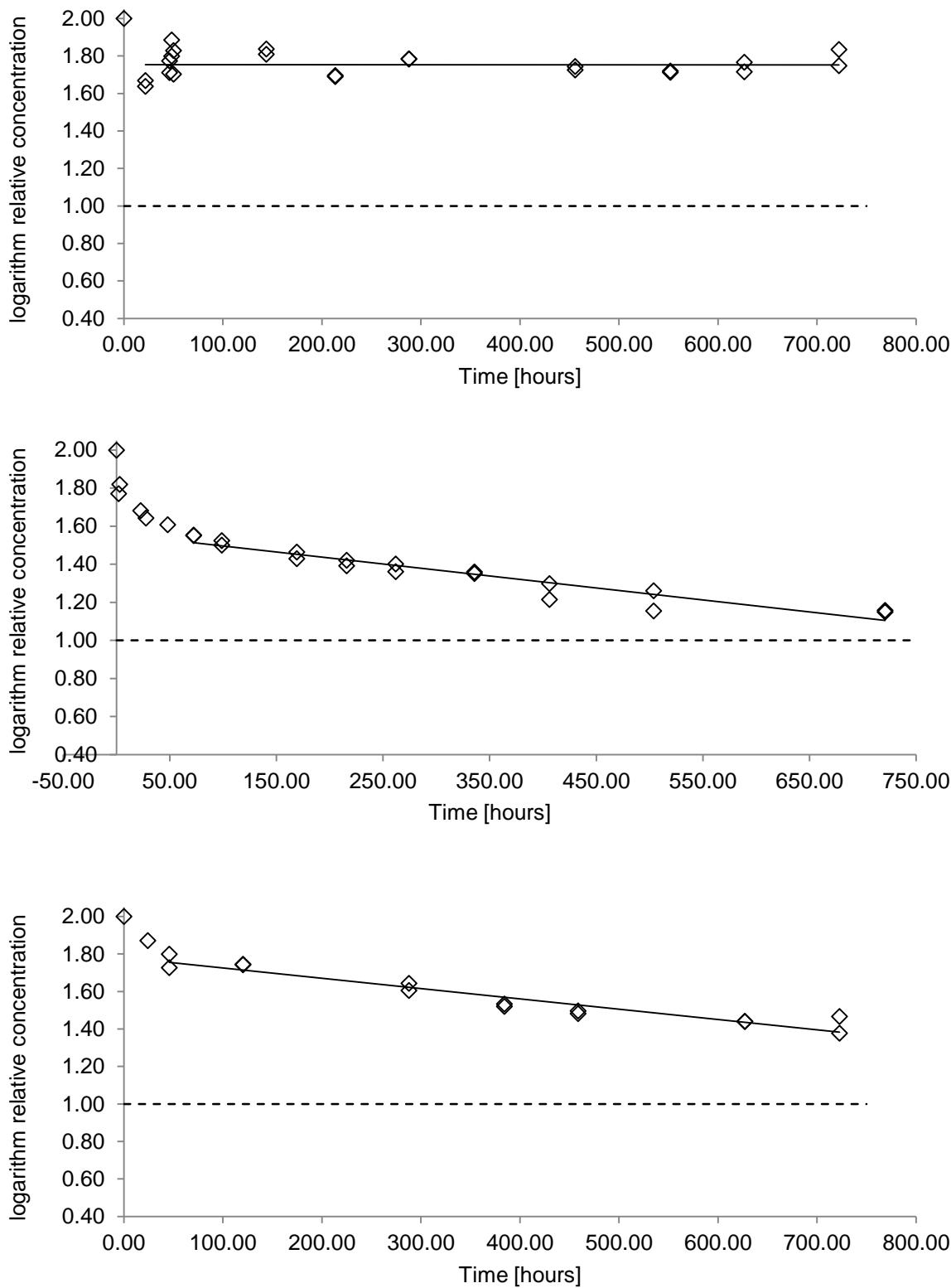
These results however are not as expected for hydrolysis. For hydrolysis, rate constants and slopes increase by a factor 2 to 3 with a 10°C increase in temperature. This was not observed. Even a negative half-life time was obtained at 20°C at pH 7 and pH 9 due to a slight increase of concentration with time which is not possible in case of hydrolysis.

Based on the relatively low recovery at start of the tests and the steep decrease in concentration during the first hours of the tests at 50 and 60°C, it is expected that the decrease in concentration observed in the various tests at pH 4, pH 7 and pH 9 is most probably due to adsorption and/or limited solubility in the buffer solutions<sup>1</sup> and not due to hydrolysis. The amide bond in the various compounds present in MLA-3202 is expected to be stable at pH 4, pH 7 and pH 9.

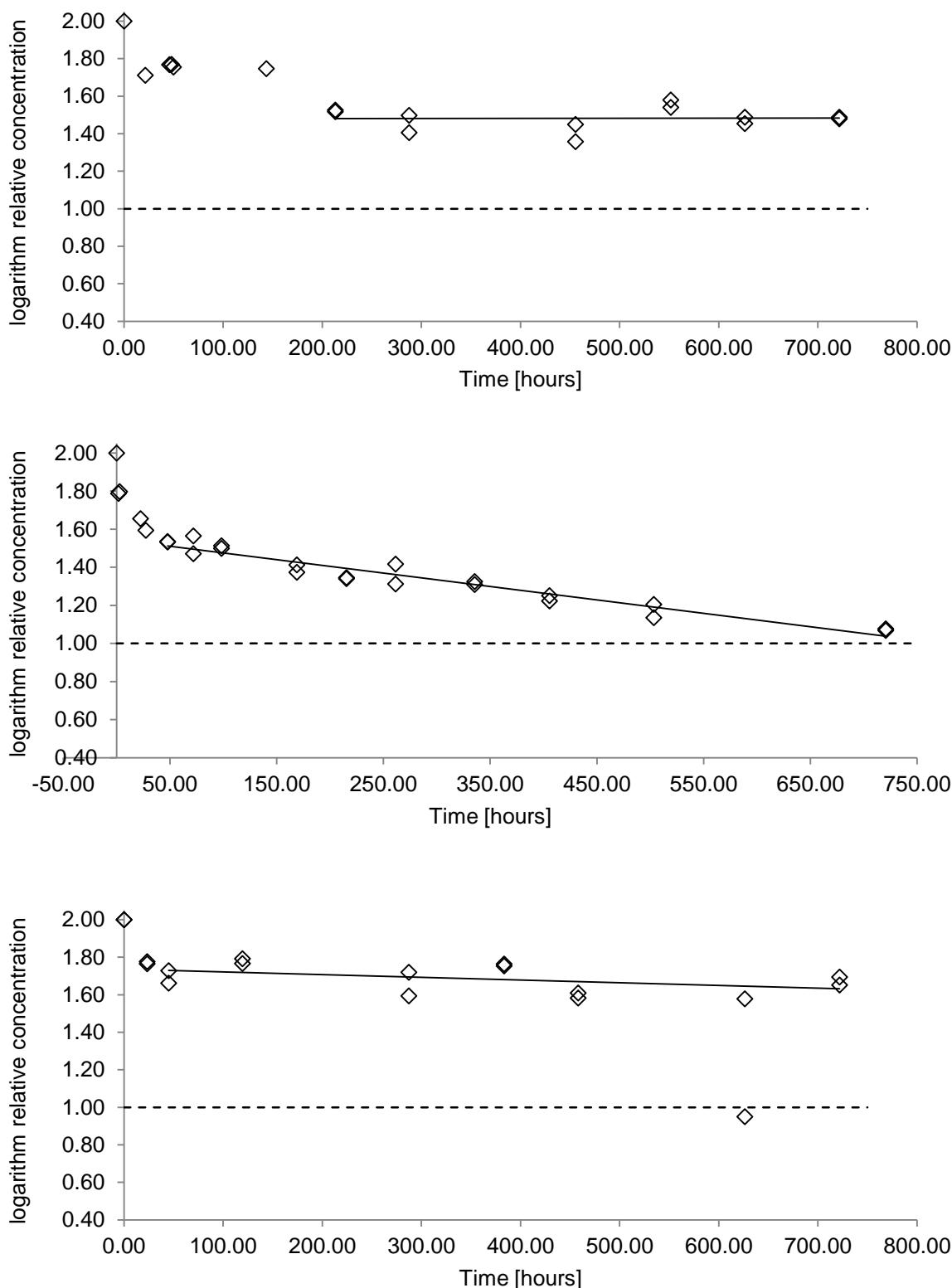
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<sup>1</sup> Water solubility was determined as part of Charles River Laboratories Den Bosch project 511870  
“Determination of physico-chemical properties of MLA-3202”

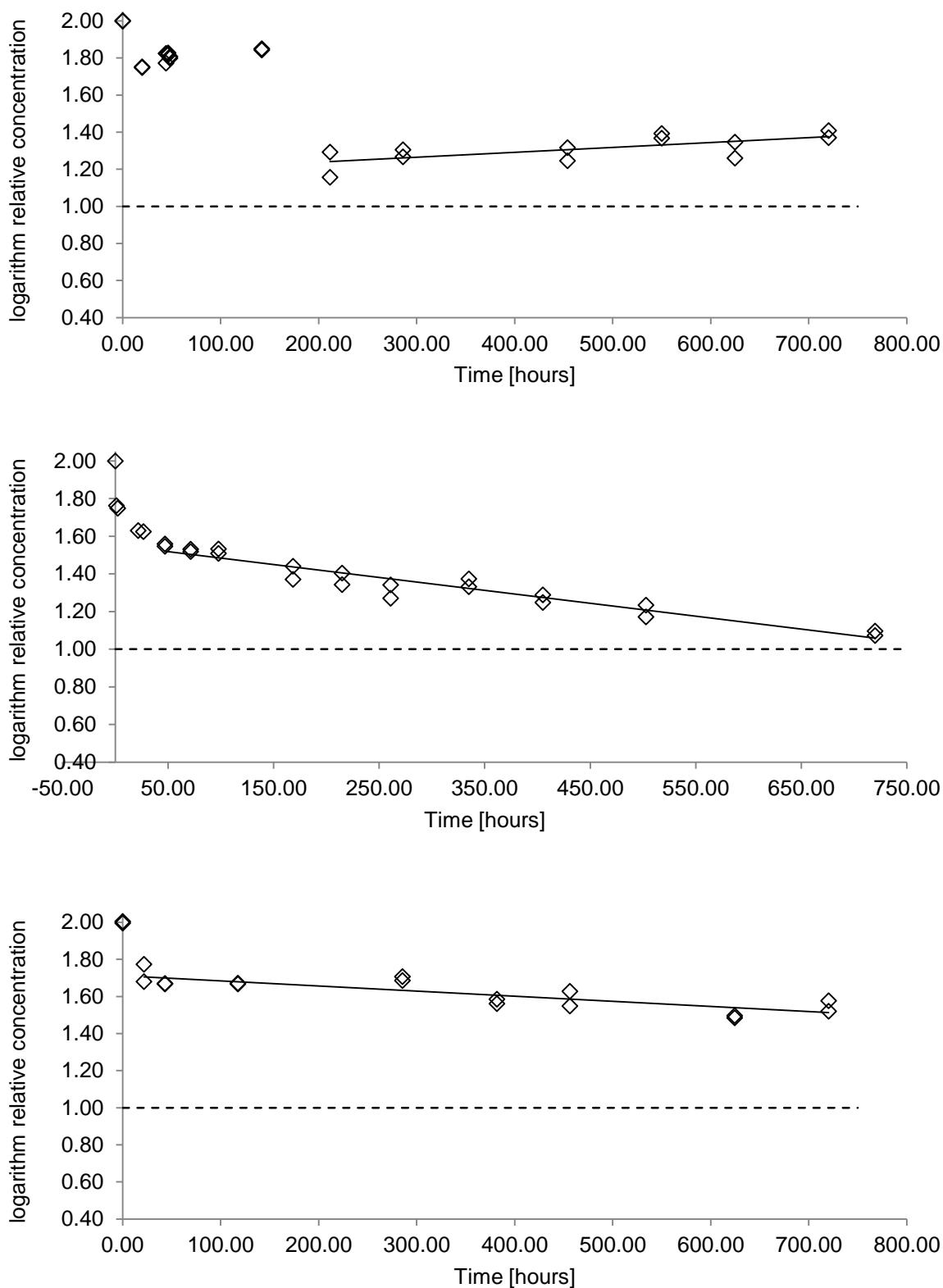
## 6.9. Figures



**Figure 1**  
**pH 4; Plot of the logarithms of relative concentration against time at 20°C [top], 50°C [middle] and 60°C [bottom].**



**Figure 2**  
**pH 7; Plot of the logarithms of relative concentration against time at 20°C [top], 50°C [middle] and 60°C [bottom].**



**Figure 3**  
**pH 9; Plot of the logarithms of relative concentration against time at 20°C [top], 50°C [middle] and 60°C [bottom].**

## 7. ADSORPTION COEFFICIENT

### 7.1. Guidelines

- European Community (EC), EC no. 440/2008, Part C: Methods for the Determination of Ecotoxicity, Guideline C.19: "Estimation of the Adsorption Coefficient ( $K_{oc}$ ) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)", Official Journal of the European Union no. L142, May 31, 2008.
- Organization for Economic Co-operation and Development (OECD), OECD Guideline for the Testing of Chemicals no. 121: "Estimation of the Adsorption Coefficient ( $K_{oc}$ ) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)", January 22, 2001.

### 7.2. Reagents

Water	Tap water purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA)
Methanol	Biosolve, Valkenswaard, The Netherlands or VWR International, Leuven, Belgium

All reagents were of analytical grade, unless specified otherwise.

### 7.3. Performance of the study

The principle of the test method is similar to that of the OECD guideline no. 117: "Partition coefficient (n-octanol/water), high performance liquid chromatography (HPLC) method". While passing through the column along with the mobile phase the test item interacts with the stationary phase. As a result of partitioning between mobile and stationary phases, the test item is retarded. The dual composition of a cyanopropyl stationary phase, having polar and non-polar sites allows for interaction of polar and non-polar groups of a molecule in a similar way as is the case for organic matter in soil or sewage sludge matrices. This enables the relationship between the retention time on the column and the  $K_{oc}$  on organic matter to be established.

The test item is a UVCB. In order to determine whether the N,N-bis(2-hydroxypropyl) tallow amide compounds in the test item are ionised for 10% or more in the pH range 5.5-7.5, the  $pK_a$  values of the C16:0 and C18:1 compounds were calculated using the Perrin calculation method (pKalc 5.0, module in Pallas 3.0, CompuDrug International San Francisco, CA, USA). The calculations showed that the N,N-bis(2-hydroxypropyl) tallow amide compounds in the test item do not have  $pK_a$  values in the pH range 1-14 and that in this pH range they are in their non-ionised form. The HPLC analysis was therefore performed without buffering of the mobile phase (neutral pH).

Solutions of reference substances with known  $\log K_{oc}$  values based on soil adsorption data and the test item were analysed. The capacity factor ( $k'$ ) of each compound was calculated from its retention time. The  $\log k'$  values of the references substances were plotted against the known  $\log K_{oc}$  values. A linear regression program was used to calculate the calibration curve. Linear regression analysis was performed using the least squares method. The coefficient of correlation ( $r$ ) was calculated. The  $\log K_{oc}$  value for the test item was calculated by substituting its mean  $\log k'$  in the calibration curve. The value of  $\log K_{oc}$  obtained from duplicate measurements was within  $\pm 0.25$  log units.

## 7.4. Analytical method

### 7.4.1. Analytical conditions

Instrument Acquity UPLC system (Waters, Milford, MA, USA)

Detector Acquity UPLC PDA detector (Waters)

Column Acquity UPLC HSS Cyano, 100 mm × 2.1 mm i.d.,  
 $d_p = 1.8 \mu\text{m}$  (Waters)

Column temperature  $35^\circ\text{C} \pm 1^\circ\text{C}$

Mobile phase A - methanol

B - water

Gradient<sup>2</sup>

Time [minutes]	%A	%B
0	55	45
10	55	45
10.1	100	0
40	100	0
40.1	55	45
50	55	45

Flow 0.4 mL/min

Injection volume 1  $\mu\text{L}$

UV detection 210 nm

<sup>2</sup> A gradient was applied in order to elute components with  $\log K_{\text{oc}} > 5.63$  from the column. The reference compounds are eluted in the isocratic part of the method and the gradient step was not applied for these injections.

### 7.4.2. Preparation of the solutions

#### Solution of the unretained compound

A 5.0 g/L stock solution of formamide (99.2%, [75-12-7], Alfa Aesar, Karlsruhe, Germany) in methanol was used. The stock solution was diluted to obtain an end solution of 55/45 (v/v) methanol/water. The formamide blank solution was 55/45 (v/v) methanol/water.

#### Reference substance solutions

Stock solutions of the reference substances at concentrations of approximately 1 g/L in methanol were used. The stock solutions were diluted to obtain an end solution of 55/45 (v/v) methanol/water. The blank solution for the mixture of reference substances was 55/45 (v/v) methanol/water.

Reference substance	Purity	CAS number	Supplier	log K <sub>oc</sub> <sup>#</sup>
Acetanilide	99.8%	103-84-4	Sigma-Aldrich	1.26
Monuron	99.9%	150-68-5	Sigma-Aldrich	1.99
2,5-Dichloroaniline	99.9%	95-82-9	Merck	2.55
Naphthalene	99.5%	91-20-3	Acros Organics	2.75
Benzoic acid phenylester	99.9%	93-99-2	Sigma-Aldrich	2.87
Fenthion	97.5%	55-38-9	Sigma-Aldrich	3.31
Phenanthrene	98.1%	85-01-8	Acros Organics	4.09
4,4'-DDT	98.7%	50-29-3	Sigma-Aldrich	5.63

<sup>#</sup> values according to the OECD 121 guideline based on soil adsorption data

Acros Organics, Geel, Belgium

Merck, Darmstadt, Germany

Sigma-Aldrich, Steinheim, Germany

#### Test solution

A 1000 mg/L solution of the test item was prepared in methanol. The test item blank solution was methanol.

### 7.4.3. Injections

The reference substance and test item solutions were injected in duplicate. Blank solutions were analysed by single injection.

### 7.5. Formulas

$$\text{Capacity factor (k') } k' = \frac{(t_r - t_0)}{t_0}$$

where:

t<sub>r</sub> = retention time

t<sub>0</sub> = mean column dead time

Calibration curve

$$\log k' = a \log K_{oc} + b$$

where:

a = slope

b = intercept

## 7.6. Results

### 7.6.1. Determination of the $K_{oc}$

Chromatograms of the test item stock solution and corresponding blank are shown in [Figure 4](#). In the chromatogram of the test solution, six major and several smaller test item peaks were observed. Peak area percentages were not calculated since compounds did not all elute in the isocratic part of the analysis.

The  $K_{oc}$  and log  $K_{oc}$  values of the major peaks are shown in [Table 21](#). The smaller peaks were observed at a retention time between 1.1 and 11.8 minutes.

The results of the HPLC method are given in the [Table 21](#). [Figure 5](#) shows the calibration curve of the log  $k'$  of the reference substances as function of log  $K_{oc}$ . The equation of the regression line was:  $\log k' = 0.315 \times \log K_{oc} - 0.850$  ( $r = 0.98$ ,  $n = 16$ ).

**Table 21**  
 **$K_{oc}$  of the test item**

Substance	$t_{r,1}$ [min]	$t_{r,2}$ [min]	mean $t_r$ (n=2)	log $K_{oc}$	$K_{oc}$
Formamide ( $t_0$ )	0.667	0.673	0.670		
Acetanilide	0.869	0.869		1.26	
Monuron	1.056	1.057		1.99	
2,5-Dichloroaniline	1.194	1.201		2.55	
Naphthalene	1.376	1.385		2.75	
Benzoic acid phenylester	1.606	1.613		2.87	
Fenthion	2.100	2.121		3.31	
Phenanthrene	2.327	2.347		4.09	
4,4'-DDT	5.529	5.611		5.63	
Test item – peak 1	5.387	5.405	5.396	5.4	$2.5 \times 10^5$
Test item – peak 2	7.014	7.046	7.030	5.8 <sup>1</sup>	$6.4 \times 10^5$
Test item – peak 3	8.288	8.333	8.311	6.1 <sup>1</sup>	$1.1 \times 10^6$
Test item – peak 4	9.866	9.923	9.895	6.3 <sup>1</sup>	$2.1 \times 10^6$
Test item – peak 5	11.684	11.685	11.685	> 6.3	> $2.1 \times 10^6$
Test item – peak 6	11.970	11.970	11.970	> 6.3	> $2.1 \times 10^6$

<sup>1</sup> Estimated value, calculated by extrapolation of the regression line.

## 7.7. Conclusion

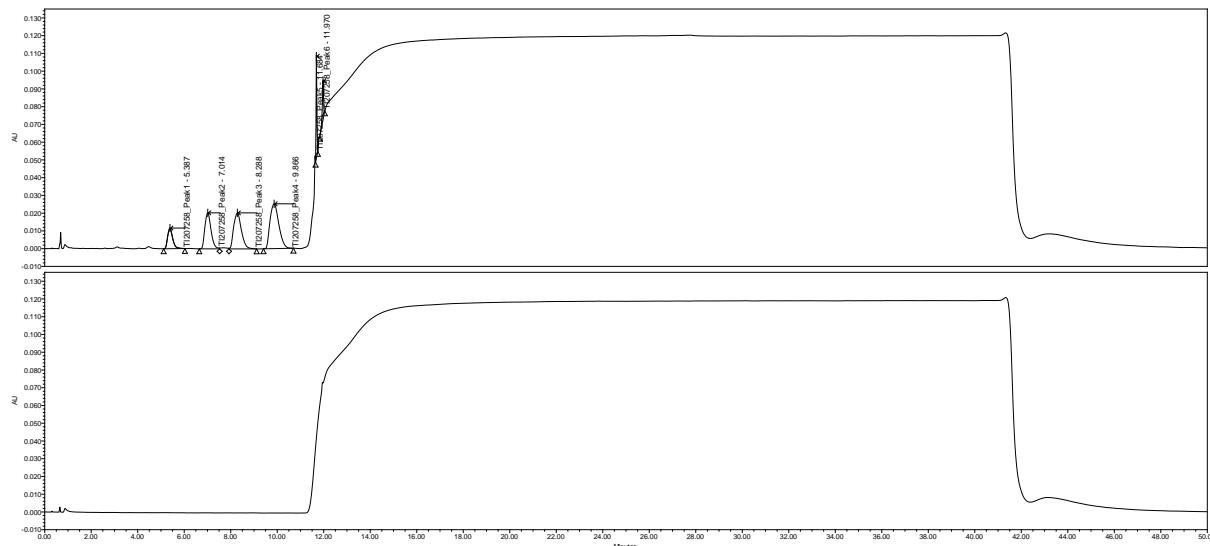
The HPLC method using soil-adsorption-reference data was applied for the determination of the adsorption coefficient ( $K_{oc}$ ) of MLA-3202.

The  $K_{oc}$  and log  $K_{oc}$  values of the test item at neutral pH were:

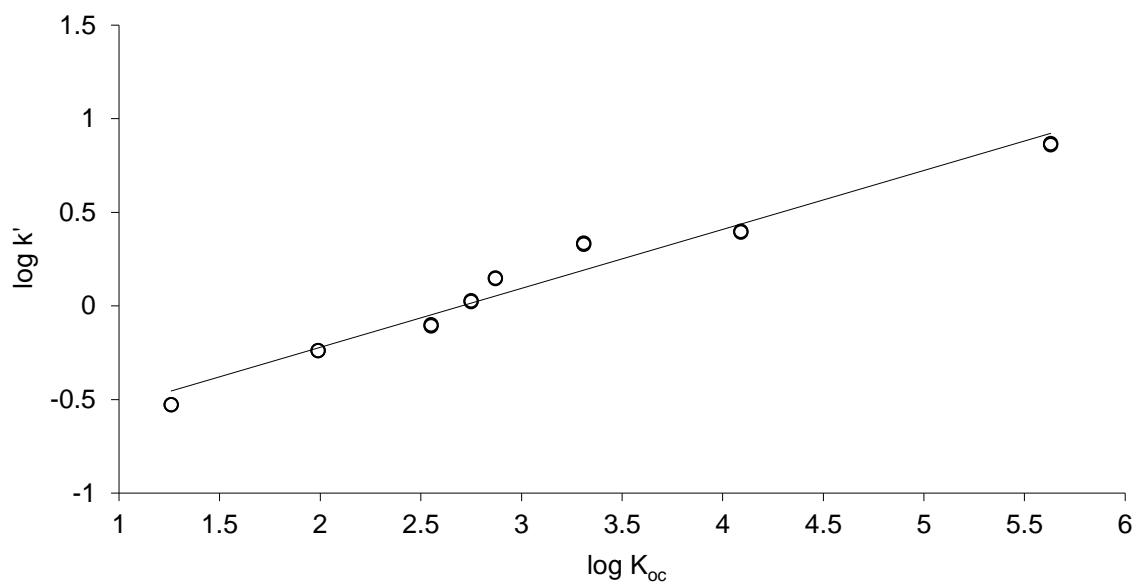
	$K_{oc}$	log $K_{oc}$
Test item – peak 1	$2.5 \times 10^5$	5.4
Test item – peak 2	$6.4 \times 10^5$	5.8 <sup>1</sup>
Test item – peak 3	$1.1 \times 10^6$	6.1 <sup>1</sup>
Test item – peak 4	$2.1 \times 10^6$	6.3 <sup>1</sup>
Test item – peak 5	> $2.1 \times 10^6$	> 6.3
Test item – peak 6	> $2.1 \times 10^6$	> 6.3

<sup>1</sup> Estimated value, calculated by extrapolation of the regression line.

## 7.8. Figures



**Figure 4**  
**UPLC chromatograms of the 1000 mg/L test item solution [top; res. id. 1249] and corresponding blank [bottom; res. id. 1252]**



**Figure 5**  
**The regression line of the reference substances: log k' versus log K<sub>oc</sub>**